FOREST INSECT AND DISEASE MANAGEMENT

Environmental Impacts of Diflubenzuron (Dimilin®) Insecticide

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INTRODUCTION

Dimilin is the trade name of the insect growth regulator diflubenzuron, N-[[(4-chlorophenyl) amino] carbonyl] 2,6-difluorobenzamide. Diflubenzuron is a non-volatile, white crystalline solid with a melting point of 230°C and a molecular weight of 310.7. It is currently registered for use in the hardwood forest to control the gypsy moth and registrations are pending for use against mosquito larvae, the cotton boll weevil, and several pests which attack soybeans. Because of its mode of action, the interruption of chitin synthesis in the insect, this compound has low mammalian toxicity, is highly selective and is environmentally safe. The purpose of this report is to provide factual information of the basic toxicology, both mammalian, and non-mammalian, the environmental fate, and the environmental interactions and safety of diflubenzuron.

Information on the environmental impacts of diflubenzuron (Dimilin®) insecticide compiled here is presented as an aid for those engaged in suppression of the gypsy moth in the Northeast.[1]

TOXICOLOGY

Mammalian toxicology

Acute studies.-- The acute toxicity of diflubenzuron has been investigated by Philips-Duphar B.V. (1973a, d, e, f, and g and 1974a, c), Huntingdon Research Center (1972, 1973a, b, 1974b, and 1975c) and Harris Laboratories (1973a and b). To best summarize this information, the data has been tabulated below:

Acute toxicity data for diflubenzuron

Animal/ formulation	*Oral* LD_{50}	*Dermal* LD_{50}	*Inhalational* LD_{50}	*Acute* IP
Mouse (tech)	4,640 mg/kg	—	—	2,150 mg/kg
Mouse (W-25)	10,000 mg/kg	—	—	—
Rat (tech)	4,640 mg/kg	—	no effect	—
Rat (W-25)	10,000 mg/kg	—	no effect	—
Rabbit (W-25)	—	4,640 mg/kg	no effect	—

In addition to the above data, diflubenzuron (40 mg technical) was a marginal eye irritant but as 50 mg in an aqueous gum tragacanth solution was not irritating. The 25 percent wettable powder was also not irritating to the eye (0.5 mg in 0.1% saline). When diflubenzuron was tested for skin irritation properties, it was found to be non-irritating as either the technical material or 25% wettable powder on both abraded and non-abraded skin.

Subchronic studies -- Diflubenzuron has also been shown to be of low subchronic toxicity to mammals (Huntingdon Research Center, 1973a, 1974a, 1975a, b, d, e, f, and g and Philips-Duphar B.V., 1973b, c). In 90-day feeding studies in the rat, a 50.0 ppm level in the diet was shown to be a no effect level while similar studies with beagle dogs showed 40 ppm to be a no effect level.

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Subacute dermal studies have been carried out with rabbits tested with 4.64% to 70% solutions of technical diflubenzuron at a rate of 1.5 ml/kg/day (Huntingdon Research Center, 1975a, and b). At the 70% level, slight to well defined erythematous reactions were observed and elevated sulfhemoglobin levels were observed with 21.4, 23.0 and 70 percent solutions. These effects were only seen with abraded skin, and weight gain, organ weight, histopathology, or cutaneous aspects of both abraded and non-abraded studies were not affected by diflubenzuron. A 10 percent solution was considered a no effect level. Subacute inhalation studies have also been carried out with little effect of treatment (Huntingdon Research Center, 1975b and d). At levels as high as 50mg/liter, one hour daily, for a three week period, dust irritation symptoms were observed at 5 and 50 mg levels but these effects dissappeared when treatment was stopped.

A number of other feeding studies have been conducted to determine the effects of diflubenzuron on reproduction, and the spontaneous tumor profile, in rats and mice, as well as to determine if diflubenzuron was mutagenic (Huntingdon Research Center, 1975g, 1976a, b). The Ames *in vitro* type assay was also used to determine the mutagenic potential of diflubenzuron (Dorough, 1976). Diflubenzuron did not effect the reproduction potential of rats exposed to levels as high as 160 ppm in the diet for three generations. At the same levels in rats and 50 ppm in mice, the compound did not show alterations in the spontaneous tumor profile and is therefore not carcinogenic. In the Ames mutagenic assay, diflubenzuron was not mutagenic with or without the microsomal activating enzyme. In studies with male mice treated with a single oral dose of 1,000 to 2,000 mg/kg, no increases in dominant lethal mutations or mutation rates were observed.

Non-mammalian toxicity

Acute studies - - Diflubenzuron, as stated earlier, has efficacy against a variety of insects of agriculture and public health importance. For this reason toxicity studies have also been carried out to determine the toxicity to species which might become exposed as a result of spray application. (Aquatic Environmental Sciences, 1976; Booth, 1976a; Booth *et al.*, 1976; Booth and Hickman, 1976; Canadian Bio-Scientific Consultants, 1975; Cannon Laboratories, 1976d and e; Hazleton Laboratories, 1973a and b, and Marine Research Institute, 1973). These acute toxicity data are found below:

Acute Toxicity to Fish and Wildlife

Avian Oral LD_{50} (Diflubenzuron)	
Anas platyrhynchos L., Mallard duck (Technical)	> 5000 mg/kg
Colinus virginianus L., Bobwhite quail (Technical)	> 5000 mg/kg
Avian 8-day Dietary LC_{50}(Diflubenzuron)	
Anas platyrhnchos L., Mallard duck (Technical)	> 4640 ppm
Colinus virginianus L., Bobwhite quail (Technical)	> 4640 ppm
Turdus musicus L., Redwing black bird (Technical)	3763 mg/kg
96-hour LC_{50}(diflubenzuron)	
Lepomis macrochirus Raf., Bluegill sunfish (Technical)	135 ppm
Lepomis macrochirus Raf., Bluegill sunfish (W-25)	660 ppm
Ictalurus punctatus Raf., Channel catfish (W-25)	> 500 ppm
Salmo gairdneri Rich., Rainbow Trout (Technical)	140 ppm
Salmo gairdneri Rich., Rainbow Trout (W-25)	240 ppm
Pimephales promelas Raf., Fathead Minnow (Technical)	320-560 ppm
Pimephales promelas Raf., Fathead minnow (W-25)	180 ppm
Micropterus dolomieui Lacepede, Smallmouth bass (Technical)	10-100 ppm

Micropterus dolomieui Lacepede, Juvenile bass (Technical)	250 ppm
Ostreidae sp., Oyster Larvae (4-days old)(W-25)	130 ppm
Daphnia magna, water flea (W-25)	1.5 ppb (48 hr.)
Gammarus pseudolimnaeus, glass shrimp (W-25)	45 ppb (48 hr.)
Chironomus plumosus, midge larvae (W-25)	560 ppb (48 hr.)
Palaemonetes pugio, grass shrimp (W-25)	640 ppb
Procambrus clarkii, red crayfish (W-25)	>500 ppb
Adonta sp. Fresh water mussel (W-25)	> 1,000 ppm
Mercenaria mercenaria quahog (W-25)	> 1,000 ppm
Physa sp. Snail	> 125 ppm
Uca pugilator, fiddler crab (W-25)	>1,000 ppm
Carcinus mainas, green crab (W-25)	>1,000 ppm
Dugesia dorotocephala, flatworm (W-25)	125 ppm
Monostyla sp. rotifier (W-25)	> 125 ppm
Cuelenterata sp., Green Hydra	> 125 ppm
Cuelenterata sp., Brown Hydra	> 125 ppm
Euglena viridis, protozoan	> 125 ppm
Paramecium multimicronucleatum, protozoan (W-25)	>125 ppm
96-hour LC_{50}(diflubenzuron Metabolites)	
Pimephales promelas Raf., Fathead Minnow (p-chlorophenylurea)	10-100 ppm
Pimephales promelas Raf., Fathead Minnow (2,6-difluorobenzoic acid)	10-100 ppm
Pimephales promelas Raf., Fathead Minnow (p-chloroaniline)	10-100 ppm
Ictaluras punctatus Raf., Channel catfish (p-chlorophenylurea)	100 ppm
Ictalura punctatus Raf., Channel catfish (2,6-difluorobenzoic acid)	100 ppm
Ictaluras punctatus Raf., Channel catfish (p-chloroaniline)	23 ppm
Chironomous plumosus (p-chlorophenylurea)	100 ppm (48 hr.)
Chironomous plumosus (2,6-difluorobenzoic acid)	100 ppm (48 hr.)
Chironomous plumosus (p-chloroaniline)	43 ppm (48 hr.)

Subchronic studies - - Although the acute studies showed that diflubenzuron was not toxic to the species of birds and fish tested, but because of its mode of action, was toxic to certain species of aquatic crustaceans and immature insects, it was necessary to determine the longterm toxicity of diflubenzuron. In a study by Cannon Laboratories (1975), Bobwhite quail and Mallard ducks were exposed to 10 or 40 ppm diflubenzuron in the diet for one laying season. Both the Mallard duck and Bobwhite quail showed no treatment related effects, and the 11 reproductive parameters measured in both the control and treatment groups were the same. With both the ducks and quail egg shell thickness, total body weight and weight gain were unaffected.

A similar reproduction study was done by Cannon Laboratories (1976a) using the Fathead minnow. The fish were exposed to five levels, 0.00675, 0.0125, 0.025, 0.05 and 0.1 ppm diflubenzuron in the water. The hatchability of the first generation eggs was the same as for the control, and both fry survival and behavior were similar to the control. The reproductive capacity of the first generation was no different from the control group and both hatchability and survival of the second generation was unaffected by the treatment.

A trout abbreviated egg to egg study was conducted at concentrations of 29 to 300 ppb in the water (USDI-FWS, 1975). Diflubenzuron had no effect on growth of the trout from the eyed-egg to the early fingerling stage.

To determine the subchronic effects of diflubenzuron on crustaceans, three studies have been carried out (USDI-FWS, 1975; Bionomics, 1975, and Cunningham, 1976). The grass shrimp *Palaemonetes pugio* has been exposed to concentrations of technical diflubenzuron of 0.7 ppb to 16.4 ppb for 35 days. At 0.7 ppb 69% of the population survived while at 1.7 ppb, only 14% of the population survived the 35-day exposure. A reproduction study using *Daphnia magna* indicated that 1.0 ppb was the lowest dose which had no effect on reproduction while 3.0 ppb was the lowest dose which caused a 20% reduction in the young produced. A similar study has also been carried out using the brine shrimp *Artemia salina.* Exposure levels of 1.0, 2.0, 5.0, and 10.0 ppb diflubenzuron were used, and the reproductive performance was determined using pair mating experiments. Reproductive life-span was reduced at concentrations of 2, 5, and 10 ppb with the females being the most sensitive. Naupliar survival was not affected at 10 ppb; however, cyst hatchability was reduced 18% over the control groups.

It is apparent that diflubenzuron, because of its unique mode of action, is non-toxic to birds and fish. The compound does, however, have an effect on some species of chitin containing invertebrates. This response to the toxic xenobiotic varies a great deal between species and is related to the exposure level, the time of exposure, the ease with which the compound is absorbed by the organism and its inherent ability to degrade the compound. Both the mode of action and the ability of a number of organisms to degrade diflubenzuron is discussed in the next section.

Biochemical aspects of toxicology

Mode of action of diflubenzuron -- Diflubenzuron is one member of a unique family of insect growth regulators which are effecacious because of their inhibitory activity in chitin biosynthesis. The inhibition of chitin synthesis is specific to insect and closely related forms but has no effect on the chitin producing mechanism of the yeast, *Saccharomyces cervisiae,* and the fungi *Neurospora crassa* and *Entomophtora thaxteriana* (Cabib, 1976, Philips-Duphar B.V., 1975a). A great deal of research has been conducted in the Philips-Duphar laboratories, as well as in a number of laboratories in the United States, in an attempt to elucidate the exact site of biochemical activity of diflubenzuron.

Results of investigations at Philips-Duphar through studies of incorporation of ^{14}C-labeled glucose into the chitin matrix (Verloop and Ferrell, 1976) showed that the effect of diflubenzuron on chitin biosynthesis occurred rapidly within fifteen minutes after application to *Pieris brassicae,* while Casida *et al.* (1977) observed an effect 3 minutes after application to the mealybug. Effects were seen in less than 80 minutes by Dr. Ker (1977) following treatment of adult locusts while no effects were observed on protein synthesis. Sowa and Marks (1975) of the USDA at Fargo, investigated chitin synthesis in a model system of cockroach leg regenerates and found that the I_{50} of diflubenzuron for this *in vitro* system is $6 \times 10^{-10}M$ while protein synthesis associated with this cuticular fraction was unaffected. This rapid response by the organism to the chemical appears due to blockage of incorporation of N-acetyl glucosamine as indicated by experiments with ^{14}C-labeled UDP-N- acetyl glucosamine. This affect on chitin synthesis occurs not only in actively developing insect larvae but also within the egg prior to eclosion. Effects have been observed through topical applications of the eggs as well as through contact with a treated female during egg deposition (Grosscurt, 1976). As with cuticular synthesis of developing larvae, inhibition of the cuticular synthesis mechanism of the developing embryo causes this ovicidal effect.

Blocking of N-acetyl glucosamine incorporation into chitin became more obvious in studies comparing the effect of the competitive chitin synthesis inhibitor, polyoxin-D, and diflubenzuron. Both compounds gave the same type of inhibition of ^{14}C- UDP-N-acetyl glucosamine incorporation; however, diflubenzuron was 100 times more active due to its greater affinity for insect tissue. It is therefore quite clear that diflubenzuron interrupts a step in which the chitin molecule is condensed from acetyl glucosamine sub-units. While polyoxin-D is a potent fungicide, diflubenzuron has no effect on the mechanism of chitin synthesis in fungal organisms. This may be explained by two theories: the enzyme may be structurally different or a penetration barrier for diflubenzuron exists in the fungi which prevents the molecule from reaching its site of action.

Although research has indicated that diflubenzuron may also be effective through stimulation of chitinase and phenyloxidase activity (Yu and Terriere, 1975). These phenomena appear to be of secondary nature occurring 2 to 3 days after treatment and during the development of toxic symptoms.

Although no structural defects in the toxicology experiments with diflubenzuron have been observed, the influence of diflubenzuron on biosynthetic pathways of structural proteoglycans (i.e., hyaluronic acid, chondroitin sulfate, etc.) are also derived from UDP-N-acetyl glucosamine. It seems unlikely due to the absence of structural defects in the toxicological studies, that there would be an influence on the enzymes that condense UDP-NAGA into hyaluronic acid and other mucopolysaccharides.

In fact, experiments carried out by Philips-Duphar B.V. (1976c), Brigham Young University (Seegmiller and Booth, 1976) and the National Institutes of Health (Hascall, 1977), indicate diflubenzuron does not affect the synthesis of these proteoglycans *in vivo* or *in vitro.*

Metabolic Fate in Animals, Aquatic Organisms, and Insects -- The previous sections indicate that the toxicity of diflubenzuron is very low for mammalian and non-mammalian species exclusive of insects and certain other chitin containing arthropods. This low toxicity is in part related to the ability of the compound to be absorbed by the animal exposed and its ability to biochemically detoxify and eliminate it from his system. These processes have been extensively investigated in the laboratory rat, poultry, cattle, sheep, fish, and a variety of insects during the development of diflubenzuron for agricultural use. These animals have been exposed to diflubenzuron by single oral or continuous dietary exposure or, in the case of the insects, topical treatment or injection.

Laboratory rats have been exposed to dual labeled diflubenzuron (1.0 mg/animal) by stomach tube and observed for 6 days (Philips-Duphar B. V., 1974c, 1975b). In this time, elimination 75-95% of the dose, while retention in the carcass was only 0-4%. Fifty percent of the dose was absorbed from the gut with approximately 20% of this material undergoing hydrolysis between the rings to yield 2,6-difluorobenzoic acid and 4-chlorophenylurea. The remaining 80% of that which was absorbed from the gut underwent ring hydroxylation and elimination via the urine or via the bile. That material which is not absorbed from the gut was not altered and passed directly through the animal.

The fate of diflubenzuron in larger animals is very much similar. In the case of both sheep and a lactating cow, absorption from the gut was approximately 50% of the administered single oral dose (Ivie, 1977). The degradative pathway is very similar with the major products being the ring hydroxylated material or fission products from cleavage between the carbonyl and amide groups. In these animals, urinary excretion accounted for 16% (cow) to 40% (sheep) of the dose, while the feces contained the remaining

radiocarbon administered. Following treatment of the cow, negligible amounts of the dose or its decomposition products were secreted into the milk and after the 7-day post treatment period only 0.2% of the dose was accounted for in the milk.

Both red and white chickens and a male swine were exposed to ^{14}C-labeled diflubenzuron (5 mg/kg) in a study carried out by the University of Maryland, (Opdyke, 1976). For both these animals, absorption was less than had been observed previously (only 5% in the swine and 10 to 20% in the chickens). The absorbed material was biochemically altered via the pathways already discussed, and, as was observed with the laboratory rat, the cow, and the sheep degradation by the gut flora did not occur and that material which was not absorbed by the gut was excreted in the feces as intact diflubenzuron. To test these results, *in vitro* experiments were conducted at College Station, Texas using ovine digestive tract fluids (Ivie, 1977). Less than 1% degradation was observed after a 24-hour incubation period had been used.

These studies using rats and large domesticated farm animals which might be exposed to diflubenzuron residues as a result of agricultural usage, illustrate further the safety of the material. The animals studies have a limited absorptive capacity and that material which is absorbed is rapidly degraded and eliminated. Further studies have been carried out to determine if a longer exposure via the diet would affect the safety of diflubenzuron or create tissue associated residues of the parent compound or its metabolites.

Cannon Laboratories (1976b, 1976c) have conducted 28-day dietary exposure studies using both lactating dairy cattle and white leghorn laying hens. The cattle were exposed to levels from 0.05 to 250 ppm ^{14}C-diflubenzuron given as a daily capsular dose based on feed consumption while the chickens were exposed to levels of 0.05 to 5.0 ppm ^{14}C-diflubenzuron given in the same manner. The animals were also given 7 to 14 withdrawal periods from treatment. In the cattle study, residues of diflubenzuron did not accumulate in the milk and at the 250 ppm exposure level only 0.2 ppm, total ^{14}C-residue, could be found in the milk at plateau. Residues of diflubenzuron or its breakdown products in the blood, fat, and muscle were nondetectable throughout the study and only the liver and kidney contained radiocarbon residues. The kidney contained residues at the high exposure level only (1.06 ppm) while plateau residue levels were 8 ppb (0.05 ppm feeding level). Radiocarbon residues in these tissues were not unexpected due to their involvement in biochemical alteration and elimination of foreign chemicals.

During the poultry study, egg production was not affected. The birds were sacrificed periodically during the study for determination of residues radiocarbon levels in the tissues and eggs. Plateau levels were observed in 7-10 days after beginning the treatment. Radiocarbon residues in the eggs were 2.0 ppb at the low (0.05 ppm) exposure level to 0.24 ppm at the high (5.0 ppm) feeding level. The body fat contained radiocarbon residues of 4.0 ppb to 0.34 ppm, the kidney contained residues of 2.0 ppb to 0.14 ppm, and the liver contained from 2.0 ppb to 0.22 ppm total radiocarbon. The muscular tissue contained non-detectable residues at the 0.05 and 0.5 ppm exposure levels and only 0.05 ppm at the 5.0 ppm feeding level. At all exposure levels, diflubenzuron equivalents which accumulated in the tissues of the chicken or in the eggs had dissipated to below detectable levels in less than one week after treatment had been stopped.

The results of these two studies indicate that the residues which would accumulate in meat, milk or eggs are extremely low and that the bioaccumulated residues are not likely to be retained and the exposure has stopped.

The fate of diflubenzuron in elements of the aquatic ecosystem has also been investigated. A 750-gallon model pond ecosystem containing pond sediment, clams, crayfish and bluegill sunfish was treated with ^{14}C-labeled diflubenzuron formulated as Dimilin W-25. Two applications were made at a rate of 0.04 lb. a.i./acre (11 ppb) at a 2-week interval. The experiment was carried out for a total of 42 days. The dynamics of diflubenzuron degradation is still being determined however, preliminary results (Nye, 1977) show that the crayfish did not absorb residues from the water or sediment. The clams contained a maximum of 20 ppb 3 days after the first treatment (which had dissipated to 4 ppb 7 days later) and these radiocarbon residues were identified as entirely parent compound. The diflubenzuron in the water dissipated very rapidly and the half-life was less than 24 hours through a combination of settling of the formulation and degradation. Only one degradation product was found, the 4-chlorophenylurea metabolite, and concentrations of this metabolite reached a plateau of 0.8 ppb 8 days after the first treatment and 3 days after the second treatment. Unlike the clams the fish retained a residue which was similar to that found in the water, however, exact identification is still underway.

As stated previously, diflubenzuron is extremely efficacious against insects and certain other chitin containing arthropods. This efficacy is extremely variable and is directly related to the ability of the insect to absorb the compound and to a lesser extent his ability to alter the parent compound biochemically. In studies with the boll weevil (Still and Leopold, 1975), the insects were exposed to ^{14}C-labeled diflubenzuron by topical application or by injection into the abdomen. Diflubenzuron was absorbed through the cuticular surface readily but was not degraded by the weevil. The distribution of radiocarbon in the insect, the frass, a cage wash, and in the diet was essentially the same after topical treatment or injection, and 13 days after treatment 8-10% remained in the weevil 20 to 24% was found in the frass, 45-66% was found in the cage wash and 3-16% was found in the food.

In studies with house flies *Musca domestica* L., and stable flies *Stomoxys calcitrans* L., (Ivie and Wright, 1977) a difference between the two species was observed in the degree of penetration, the degree of bioalteration, and the amount secreted into the eggs. The stable fly had lesser ability to degrade the parent compound than did the house fly and subsequently deposited more of the compound within the eggs. This caused greater efficacy to be observed in the stable fly as the difference in the amounts found in newly deposited eggs were ten-fold. The degree of metabolism which did occur was only 1% in the case of the stable fly, and 8% in the case of the house fly.

It is clear that diflubenzuron is rapidly degraded, the residue eliminated from a number of domesticated animals and fish, and does not accumulate within these species. The insect on the other hand does not possess the mechanism needed to detoxify diflubenzuron and because of his sensitivity is susceptible to extremely low levels once the material has entered his system.

FATE IN THE ENVIRONMENT

Persistence in soil, water hydrosoil, and plants-aquatic vegetation

Soil-- In laboratory experiments, the half-life of diflubenzuron was shown to be 0.5 to 1.0 week. This rapid degredation was unrelated to soil type but was shown to be very much dependent on both the microbial activity of the soil and the particle size of the diflubenzuron. (Philips-Duphar B.V., 1973h, 1974d, 1975c). When sterile soil was tested 95% of the parent compound remanined after one year. With particle sizes of ten

microns the half-life in soil was found to be greater than 21 weeks while when two micron particles were applied to the soil (the mean particle size of formulated diflubenzuron is two microns) the 0.5 to 1.0 week half-life was observed. The degradation products in the soil are 4-chlorophenylurea, which contributes to soil bound residues, and 2,6-difluorobenzoic acid which is degraded rapidly to carbon dioxide and water. The half-lives of the 4-chlorophenylurea and 2,6-difluorobenzoic acid are 2-3 months and 4 weeks respectively.

Under field conditions studies with radiolabeled diflubenzuron indicate that the degradation can be even more rapid (Analytical Development Corporation, 1976c). Following soil incorporation the total radiolabel had a half-life of $<$4 days and diflubenzuron could not be detected after 24 hours.

As stated previously, the short persistence of diflubenzuron is in part dependent on soil microbial activity. Studies at Brigham Young University (Pintar *et al.,* 1975) have shown that some 111 soil bacteria could utilize diflubenzuron as a sole carbon or sole carbon and nitrogen source and growth of a number of other bacteria was not influenced at concentrations as high as 10^5 ppm.

Water-hydrosoil systems. -- The persistence of diflubenzuron in water and soil-water systems is, as with oil alone, related to the microbial activity and the particle size of the material applied (Philips-Duphar B.V., 1973h, 1974d, 1975c). As with agricultural soils the half-life in hydrosoils is 0.5 to 1.0 weeks for the parent compound and 8 weeks for the entire radiocarbon residue.

The half-life of diflubenzuron in water is even shorter. A study by the University of California (Schaefer and Dupras, 1975) has shown this half-life to be less than 24 hours and to be related to pH, temperature and suspended organic matter in the water. At increased temperature and pH, degredation is more rapid and in the presence of suspended organic matter, the diflubenzuron is tied up and unavailable to the biological system. Using a simulated pond, under actual field conditions (Nye, 1977) the rate of degradation of ^{14}C-diflubenzuron was rapid (half-life less than 24 hours). The only detectable degradation product in the water was 4-chlorophenylurea which reached levels of 1 ppb 3-4 days after treatment of the pond at 11 ppb with diflubenzuron.

Fate in plants and aquatic vegetation. -- Because diflubenzuron is not susceptible to surface photodegradation it is quite stable following application to leaf surfaces (Philips-Duphar B. V., 1974b, Cotton Insects Research Laboratory, 1976a, Analytical Development Corporation, 1976a). Absorption and translocation was also minimal from topical application to several crop species including cotton, soybeans, corn, cabbage, and apples. Dissipation on the leaf surface is primarily a function of growth dilution and mechanical removal and 30 to 60 days after treatment, as much as 90% of the remaining material on the leaf surface was unchanged diflubenzuron.

When plants are grown in soil which has been treated with diflubenzuron, little translocation occurs and the residue which does occur in the plants is the degradation product 4-chlorophenylurea or is derived from the CO_2 released from the soil during the degradation of diflubenzuron by the soil microbes (Still, 1976; Analytical Development Corporation, 1976b; Philips-Duphar B. V. 1976b). The residues which are picked up from the soil are less than 1.0% of what was applied to the soil. Aquatic plants, both vascular and non-vascular algal forms, exposed to diflubenzuron in the water absorb the compound very rapidly (Philips-Duphar B. V., 1976a; Booth *et al.*, 1975a). Unlike terrestrial plants, the aquatic plants have the capacity to hydrolyze the parent compound and eliminate

both the parent and its hydrolysis products into the water. Thus, after an initial accumulation to levels which are greater than the surrounding water, the level of diflubenzuron in the aquatic plants had decreased within 2-4 days to a concentration below that in the water.

Mobility of diflubenzuron

Laboratory studies. -- The mobility of diflubenzuron on soil thin-layer plates using five soil types has been compared to the bench-mark chemical, diuron and two other agricultural chemicals, triphenyltin hydroxide and phenthoate (Helling, 1975). Diflubenzuron mobility was greater than the tin compound but less than phenthoate and much less than diuron in the five soil types.

To test the leaching potential of diflubenzuron, an unaged study was carried out by the University of Kentucky (Rieck, 1975). The compound was applied to the top of 20-inch soil columns of four soil types, an agricultural sand, sandy loam, salt loam, and a clay loam. Leaching did not occur beyond the top four inches after 20-inches of water was passed through the columns and only 4-chlorophenylurea was found in the leachate.

In an aged leaching study conducted by Brigham Young University (Booth *et al.*, 1975b) at levels of 0.04 and 2.0 ppm ^{14}C-Dimilin W-25 diflubenzuron was shown to be nonvolatile during the aging process. However, during this degradation process, $^{14}CO_2$ evolution was 2.4 and 20% of the applied material for the low and high rates respectively.

After this aging process, when the soil was placed on the top inch of a 12-inch soil column, the ^{14}C-residues of diflubenzuron did not leach below the top two inches and only 2-10% of the radiocarbon was found in the leachate. The extractable material isolated from the soil sections was primarily parent material (84%).

A laboratory run-off study was also conducted (Bio-Search and Development, 1975) to determine the run-off and lateral movement potential of diflubenzuron. When 0.75 inches of rainfall was applied to 4-foot square by six-inch inclined bed after Dimilin W-25 had been incorporated into the top 3 inches of soil (1.0 lb a.i./acre) in the upper half of the soil bed no diflubenzuron (<0.01 ppm) could be detected in the run-off water. The residues of diflubenzuron did not leach into the bottom 3 inches of soil and no residues had moved into the lower third of the soil bed.

Field studies. -- A field study with radiolabeled diflubenzuron was conducted by the Analytical Development Corporation (1976c) to investigate the degradation and leaching of diflubenzuron in an agricultural soil under field conditions. After incorporation into the top 3 inches of soil, the half-life for diflubenzuron was less than 48 hours while the half-life for total radiocarbon in the soil was 4 days. Only 0.003 ppm was found in the 3-6 inch soil depth at 4 weeks after application and this had decreased to less than 0.001 ppm 16 weeks after incorporation at rates of 0.12 lb. a.i./acre (0.3 ppm).

Bioaccumulation of diflubenzuron

Aquatic systems. -- Both rainbow trout and bluegill sunfish have been exposed to levels of 0.01 and 1.0 ppm of ^{14}C-labeled diflubenzuron in a static system (Booth *et al.*, 1976b). During the 20-day exposure period, radiocarbon levels in the fish reached levels 8-10 fold higher than the water in the 1.0 ppm tank and 30 fold higher than water in the 0.01 ppm tank. Plateau levels in the fish were reached within 2 days (0.01 ppm tank) or 6-9 days (1.0 ppm tank).

Following the 20-day exposure period, the fish were removed from the treatment tanks and placed in diflubenzuron-free water. The residues quickly dissipated from the fish and after 5 days 90 to 98% of the radiocarbon had been eliminated from the bluegill sunfish. Roughly 65-93% of the radiocarbon was eliminated from the trout in 15 days. The ^{14}C-residues in both the water and fish were analyzed by comparative thin-layer chromatography and found to be a mixture of diflubenzuron and its degradation products 4-chlorophenylurea, 2,6-difluorobenzoic acid and 4-chloroaniline.

It should be emphasized here that a static system does not reflect an actual field situation in which the rate of degradation of diflubenzuron is extremely rapid and the absorption of the parent compound and its degradation products to the soil and suspended organic matter is extensive and would decrease it bioavailability.

Water-hydrosoil systems. -- Both the catfish and crayfish have been exposed to residues of diflubenzuron associated with soil (after it had been aged both aerobically and anaerobically) to better estimate the bioaccumulation potential of this insect development inhibitor (Booth, 1975; Booth *et al.*, 1975b,c). After the soil had been treated at levels of 0.01, 0.5 or 1.0 ppm and aged, it was flooded and the crayfish or catfish added. Because the diflubenzuron residues have a great affinity for the soil the degree of bioaccumulation was extremely low and plateau radiocarbon levels were between 0.004 to 0.016 ppm for the two species. This plateau was reached 3-7 days after addition of the fish. Only 2-3 percent of the radiocarbon added to the soil could be found in the water.

The potential for bioaccumulation of diflubenzuron from an aquatic system exists because the octanol-water partition coefficient of the compound is high (approximately 5,000). The bioaccumulation under actual use conditions will vary with both the rate of degradation and the affinity for the hydrosoil that exists with diflubenzuron. In most cases, the biomagnification for total diflubenzuron equivalents will be less than 50-fold greater than that of the water, where with a number of agricultural chemicals no longer in use, these factors can be as high as 30,000 or higher.

ENVIRONMENTAL INTERACTIONS

The forest ecosystem

Diflubenzuron formulated as the 25% wettable powder (Dimilin W-25) is currently registered with the Environmental Protection Agency for use against the gypsy moth *Lymantria dispar* L. This formulation is also under investigation for control of a number of other forest insects, among them the spruce budworm *Choristoneura fumiferana* (Clem.), Douglas-fir tussock moth *Orgyia pseudotsugata* (McDunnough), and the forest tent caterpillar *Malacosoma disstria* (Hubner). In cohabitation with these target insects are a number of non-target species which have been investigated to determine the effects of application on these elements of the forest ecosystem (USDA, 1975, Simmons, 1975a and 1975b; Brown and Diamond, 1976; Low, 1975; Elliott, 1975; Buckner *et al,* 1975; Matthenius, 1975).

In these extensive environmental impact studies, several types of forest ecosystems were treated with Dimilin W-25 at rates from 0.06 to 0.3 lb. a.i./acre. Following application, elements of the forest ecosystem including the soil microbes and invertebrates, terrestrial insects including predators and parasites of the target species, aquatic insects and other non-target crustaceans, fish, small forest mammals, and birds, were monitored for the effects of treatment.

No treatment related effects were observed with elements of the soil community, including soil microbes and fungi, soil inhabiting mites, and collembolans. It was shown that diflubenzuron had no effect on the organisms that are involved in degradation and utilization of the forest leaf litter. In the studies with the terrestrial insects, the single application of diflubenzuron had no effects on the free flying, forest-inhabiting insects. Honey bees were unaffected when hives were placed directly within the test areas. The effects monitored were honey production, egg production by the queen, and brood hatch development and survival. In addition, the predators and parasites which survive on the target insect were monitored. No effects were observed with predatory insects (hymenoptera and diptera) while the effects on the parasitic forms were inconsistent due to population variability and host availability.

Because of the single application used in the forest environment and the low use rate, the impact on the aquatic community was non-detectable at all trophic levels. This was also the case with the higher elements of the terrestrial forest ecosystem. Even though a potential exposure to insectivorous small mammals and birds was possible, no treatment related effects were observed. The species composition was unchanged as was the territorial distribution of the species monitored.

The use of diflubenzuron for forest insect control by single application at varying rates is extremely safe to the elements of this diverse and important non-target community.

The aquatic ecosystem

Diflubenzuron also shows good efficacy against a number of dipterous insects which have larval stages which inhabit the aquatic ecosystem. Mosquitos are the most important of these, however, research is continuing on chironomid midges, the Clear Lake gnat, and black flies. Because the application for control of these species is made directly to the aquatic habitat and diflubenzuron is a chitin inhibitor, a number of aquatic non-target species could be affected by the treatment.

A number of studies have been done under actual field conditions to estimate the impact of diflubenzuron against aquatic organisms. In a study conducted by L.S.U. (Farlow, 1976), Dimilin W-25 was applied to a coastal marsh six times over an 18-month period at a rate of 0.02 lbs a.i./acre. Some 72 species of non-target organisms were monitored throughout the study and the treatment affected the population numbers of only 5 of these species. The remaining 67 species showed population increases or no treatment related effects of the repeated application. The six applications of Dimilin over an 18-month period did influence the ecological balance in the coastal marsh community. However, the data obtained was not adequate to provide explanations of the biological significance, if any.

In a study conducted by Brigham Young University, Frost *et al.* (1976) studied the environmental effects of 8 applications of both the 25% wettable powder and a 1% granular formulation at 2-week intervals at a rate of 0.12 1b. a.i./acre (3x field use rate.) The experimental site was a fresh water marsh and the marsh bird population was extensively investigated to determine the effects on nesting behavior and reproductive success In addition, the benthic organisms and the phytoplankton communities were investigated to determine the effects of treatment on these elements of the aquatic ecosystem. For 7 species of marsh birds (Double-Crested Cormorants, White-faced Ibis, Great Blue Herons, Snowy Egrets, Black-Crowned Night Herons, Yellow-headed and Red-winged blackbirds), no effects were observed. Numbers of nests, young per nest, numbers of young fledged and species productivity were monitored throughout the nesting period. There were also

no treatment related effects on the benthic organisms (primarily oligochaetes and chironomids) as well as the phytoplankton species including blue-green algae.

A number of other studies have been carried in aquatic habitats to determine the effects of diflubenzuron on aquatic insects and non-target crustaceans. (Mulla *et al.,* 1975; Steelman *et al.,* 1975; and Minura *et al.,* 1975). Diflubenzuron has been found to reduce populations of certain sensitive non-target crustaceans, primarily water fleas, cyclops and immature copepods, as well as certain species of aquatic insects (mayflies, corixids, and notonectids).

The effect on the aquatic environment is one which is extremely variable, and although the species diversity in this habitat is often altered, populations of the non-sensitive forms adjust the overall community numbers to counteract the effect. The limited environmental impact, because of the non-persistence of diflubenzuron, is therefore short-lived and population recovery of the more sensitive species occurs within 14 to 28 days in most cases.

Agricultural ecosystems

Diflubenzuron also has activity against a variety of pests important to soybean and cotton production. It has excellent efficacy as a larvicide against the velvet bean caterpillar, Mexican bean beetle and green cloverworm in soybeans and as an ovicidal agent against the cotton boll weevil. Within these agricultural mono-cultures exist a number of non-target beneficial insects which themselves act to keep the populations of noxious insects at a reduced level, but rarely hold the level of damage to an acceptable limit without the use of agricultural chemicals. A number of experiments have been carried out in the laboratory and under field conditions to determine the effect of 1-10 weekly foliar application at rates of 0.03 to 0.25 lbs a.i./acre during the season on populations of these beneficial arthropods.

In the case of the laboratory trials (Wilkinson *et al.,* 1976; and Lyon and Lyon, 1975) diflubenzuron has been shown to have no effect on adults of several species including *Apanteles sp. Voria sp. Geochoris sp. Hippodamia sp. Chrysopa sp. Stenophorus sp. Aphelinus sp.* and *Ephistrophe sp.* Larvae of a few of these forms were affected when they were fed a treated prey species and the internal parasites were affected when the immature forms in which they live were killed by diflubenzuron.

In the case of field studies in the soybean, agroecosystem (Todd 1974, 1975, and 1976, and Turnipseed, 1975a, and 1975b), Dimilin W-25 was evaluated at rates of 0.06 to 0.5 1b. a.i./acre. Although no direct mortality was observed against spiders, nabids, geocorids and other predatory insects, slight population reduction was observed due to the decreased food supply.

In the cotton monoculture the studies on non-target beneficial insects have been quite extensive due to the variability in cultural practice with respect to the number of applications necessary to control the boll weevil (Keever, 1976, Nemec Agricultural Service 1976, and the Cotton Insects Research Laboratory, 1976b). In these trials, rates of 0.03 to 0.125 1b. a.i./acre Dimilin W-25 were applied on a weekly spray schedule for a total of 8 to 10 treatments. During the study period, beneficial insect surveys were made to determine the effects on population numbers and dynamics. These results were then compared to similar data collected from an untreated field or one treated with a mixture of toxaphene, methyl parathion and chlordimeform. In all cases, the application of diflubenzuron had no effect on populations of ladybird beetle larvae and adults, hymenopterous predators, *Chrysopa sp. Geochoris sp.* and *Hippodamia sp.* throughout

the treatment period. Both population numbers as well as the dynamics and interactions with prey species were similar to the control area. The plots treated with the standard chemical showed complete population suppression of the non-target species and it became quite clear that diflubenzuron is less environmentally harsh than chemicals which are currently registered for use in this agricultural ecosystem.

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